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Submerged Culture of the Mycelium of Various Species of Mushroom

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Submerged culturing of mushroom mycelium was reported by Humfeld several years ago (1948) and subsequently developed by Humfeld and Sugihara (1949, 1952) to permit low-cost, large-scale production.

The report of Block *et al.* (1953) that a strain of *Agaricus blazei* is suited to submerged culture indicates a continuing interest in this method of propagating mycelium. They reported excellent yields of mushroom mycelium grown on orange juice, citrus press water, and chemically defined media. Szuecs (1953) recently has suggested the use of penicillin vats for the production of mushroom mycelium with sulfite liquor as the medium.

The ability of selected stocks of various mushroom species to grow well in submerged culture is described herein. During our studies, a total of 20 species were grown successfully in submerged culture. All of the mushroom mycelium cultures were obtained from tissue culture. The sporophores were collected by the authors in the general vicinity of this laboratory, with the exceptions noted in table 1. Using standard aseptic methods, small pieces of sporophore tissue were placed on potato-dextrose agar plates. Incubation was at 28 C. Subsequent subculturing produced pure cultures of mushroom mycelium. Table 1 shows the wide variety of mushroom species that were grown by submerged culture in chemically defined medium (Humfeld and Sugihara, 1949).

A number of the stocks produced "secondary spores" (Kligman, 1942) and propagated rapidly in shaken flasks, whereas others grew in the pellet-like colonies at a much lower rate. The production of "secondary spores" is correlated with and may be necessary for the rapid propagation of mushroom mycelium. These spores occur either terminally or intercalarily (figures 1 and 3). In a 150-liter fermentor with 5 per cent inoculum, extensive sporulation of the strains of *Agaricus campestris* started approximately 15 hours after inoculation. A short while after sporulation commenced, numerous spores were observed to be in the process of germination (figures 2 and 4). "Secondary spores" were observed in all cultures that displayed the characteristic dispersed type of growth. The mode of sporulation was identical in all cases.

The main problem at present is the development in submerged culturing of "mushroom flavor" of sufficient intensity and consumer acceptability. Samples of mycelium of *Agaricus campestris* (Strains M5 (NRRL 2334) and M16 (NRRL 2335)) grown in chemically defined media were cooked and submitted to a panel of tasters. The taste panel reacted favorably though variably. The more favorable comments were that the samples were nut-like, cheese-like and pleasant. On the other hand, certain individuals could identify and did prefer samples of natural mushrooms even though the samples were disguised. Mycelium grown in solid media possesses typical mushroom odor, but the odor is not detectable in mycelium grown in submerged culture. Of the species other than *Agaricus campestris*, only *Lepiota rachodes* had a pleasant flavor.

When the problem of mushroom flavor is solved, an

[1] Deceased, March 1953.

TABLE 1. *Mushroom species grown in submerged culture on synthetic medium**

SPECIES	CULTURE NO.†	SOURCE‡	GROWTH CHARACTERISTICS	YIELD
				g dry matter/ 100 g glucose
Agaricus campestris (white var.)	M5; NRRL 2334	F	Dispersed, spores present	55–60
Agaricus campestris (cream var.)	M16; NRRL 2335	H	Dispersed, spores present	40–50
Agaricus campestris (white var.)	M28; NRRL 2336	G	Dispersed, spores present	45–55
Agaricus placomyces	M86	A	Dispersed, spores present	50–60
Agaricus rodmanii	M21	B	Pellet-like colonies	30–40
Armillaria mellea	M6	E	Dispersed, spores present	50–60
Cantharellus cibarius	M83; NRRL 2370	A	Pellet-like colonies	20–30
Collybia umbulata	M82	A	Pellet-like colonies	20–30
Collybia velutipes	M70; NRRL 2367	D	Pellet-like colonies	30–40
Coprinus comatus	M46	B	Dispersed, spores present	40–50
Coprinus comatus	M67	D	Dispersed, spores present	40–50
Hebeloma sinapizans	M84	A	Pellet-like colonies	20–30
Lepiota naucina	M73; NRRL 2368	C	Pellet-like colonies	40–50
Lepiota procera	M44	B	Pellet-like colonies	40–50
Lepiota rachodes	M76	A	Dispersed, spores present	40–50
Lycoperdon umbrinum	M85; NRRL 2372	A	Pellet-like colonies	20–30
Morchella sp.	M34	C	Pellet-like colonies	40–50
Morchella crassipes	M37; NRRL 2369	C	Pellet-like colonies	40–50
Pleurotus ostreatus	M69; NRRL 2366	B	Pellet-like colonies	40–50
Polyporus sulphureus	M72	D	Pellet-like colonies	30–40
Psilocybe sp.	M77	A	Pellet-like colonies	30–40
Schizophyllum commune	M71	D	Pellet-like colonies	30–40
Tricholoma nudum	M81; NRRL 2371	A	Dispersed, spores present	40–50

* Medium composition Humfeld and Sugihara (1949).
† Stocks designated by NRRL are in the Culture Collection of the Northern Regional Research Laboratory, Peoria, Ill.
‡ Source A. Collected by authors.
B. Mr. S. Bellici, San Francisco, Calif.
C. Dr. J. W. Sinden, Penn. State College, Pa.
D. Dr. R. Davidson, Bur. Plant Ind., Beltsville, Md.
E. Dr. P. A. Ark, Dept. Plant Path., U. of Calif., Berkeley, Calif.
F. Cave Mushroom Co., Santa Cruz, Calif.
G. Mr. G. Kennedy, Redwood City, Calif.
H. Dr. I. J. Hutchings, H. J. Heinz Co., Pittsburgh, Pa.

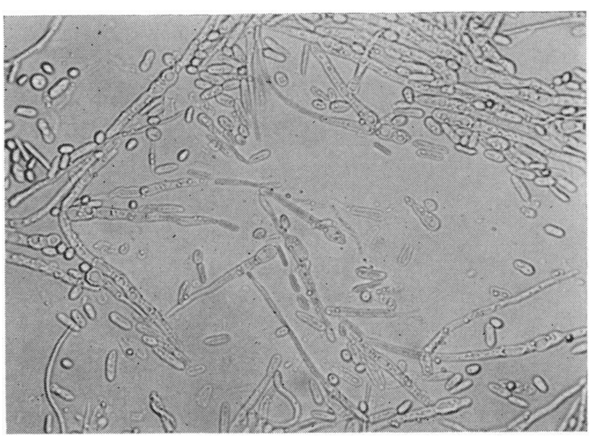

FIG. 1. *Agaricus campestris* (M5; NRRL 2334) mycelium showing terminal and intercalary spores. (× 600)

entirely new source of food supply rich in proteins, carbohydrates, and vitamins (Humfeld and Sugihara, 1949) will be available. Other possible products from mushroom mycelium propagation are antibiotics

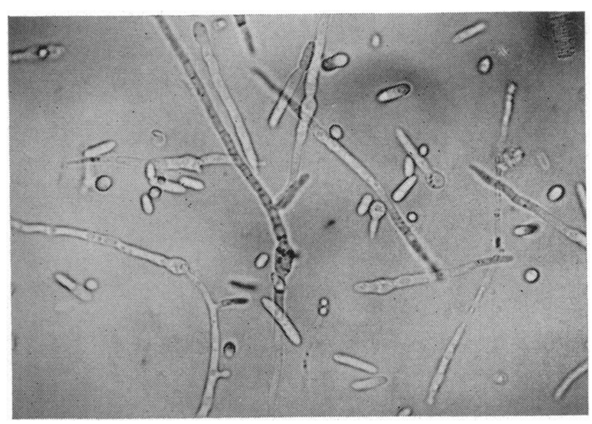

FIG. 2. *Agaricus campestris* (M5; NRRL 2334) germinating spores. (× 600)

(Robbins *et al.*, 1947) and vitamin concentrates (Block *et al.*, 1953).

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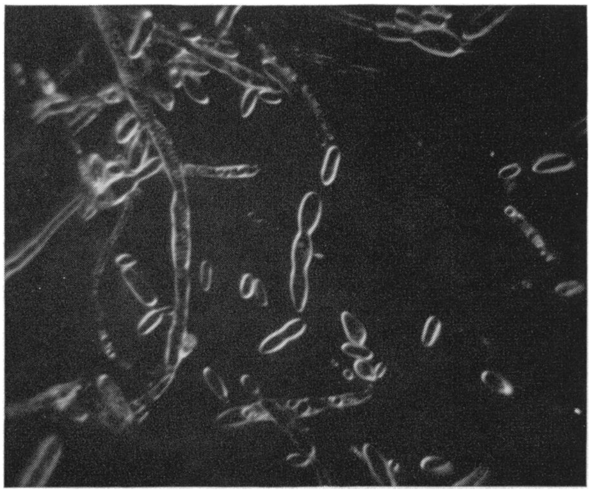

FIG. 3. *Armillaria mellea* (M6) mycelium showing terminal and intercalary spore formation. (× 1350)

FIG. 4. *Armillaria mellea* (M6) germinating spores, (× 1350)

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